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GRADUATE SCHOOL OF OCEANOGRAPHY

UNIVERSITY OF RHODE ISLAND

Reference No. 63-3

SPECTRAL SENSITIVITY AND PHOTOTAXIS IN THE OPOSSUM SHRIMP, NEOMYSIS AMERICANA SMITH

By

Sidney S. Herman

Technical Report No. 4

KINGSTON, RHODE ISLAND

Office of Naval Research
Contract Nonr-396(02)
NR 104-100

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SPECTRAL SENSITIVITY AND PHOTOTAXIS IN THE OPOSSSUM SHRIMP, NEOMYSIS AMERICANA SMITH [1, 2]

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Few studies have been conducted on photoreception in the Mysidacea. Hess (1910) and Beeton (1959) have both worked on the spectral sensitivity of mysids, but neither has subjected animals to various colors of the spectrum while controlling the intensity of light.

There is no information available in the literature on photoreception in *Neomysis americana*. Hulburt (1957) has shown that their vertical distribution in Delaware Bay is a direct result of light intensity.

This report presents methods and results of laboratory studies on spectral sensitivity and phototaxis in the opossum shrimp, *Neomysis americana* Smith.

The results of a complementary study of the vertical migration of this animal in Narragansett Bay, Rhode Island, will be reported in a separate publication. For purposes of this paper it is sufficient to state that the pattern of vertical migration was similar to that described by Cushing (1951) as typical of many zooplankton species: (1) ascent towards the surface from the day-depth, (2) departure from the surface at or before midnight, (3) return to the surface just before dawn, and (4) sharp descent to the variable day-depth when sunlight starts to penetrate the water. Light was found to be the most important single factor responsible for the migration of these animals.

Materials and Methods

Spectral sensitivity

Although there are many references to the effect of different parts of the spectrum on animal distribution, few have been made where light intensity has been controlled. In order to study the effects of various parts of the spectrum on *N. americana*, it was necessary to keep the light intensity uniform. The procedure adopted was to calibrate a model #846 Weston photronic cell against an Eppley thermopile. The thermopile measures light as a linear function, and therefore is equally sensitive to energy from all parts of the spectrum.

With calibration complete, it was possible to use the photoelectric cell to measure light intensity. The calibration was carried out at the Eppley Laboratories, Newport, Rhode Island, with the following equipment: a Leeds and Northrup model 9835-A stabilized DC microvolt amplifier, a Tinsley photocell

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galvanometer amplifier type 5214, a Kipp model AL-1 portable galvanometer, an Eppley thermopile #2427 (8 junction, Bi-AS circular, lampblack receiver of sensitivity 0.111 mμ, an Eppley microvolt comparator, and a #846 Weston photronic cell.

The photocell and the thermopile were exposed in turn to the radiant flux through Corning narrow-band interference filters and the outputs compared. The energy intensities of the different light beams were then equalized, as far as possible, with Wratten gelatin neutral density filters, and photocell outputs were determined to insure approximate equality of energy flux.

TABLE I

Calibration of photometer with Corning glass color filters

Filter	Photometer μv.	Thermopile μv.	Energy μv. cm.$^{-2}$	Photometer sensitivity μv./μw. cm.$^{-2}$
Corning				
1–02 (546–559 mμ)	360	.37	3.3	109
1–05 (515 mμ)	500	.63	5.7	88
5–77 (610 mμ)	360	.51	4.6	78
5–75 (460 mμ)	40	.43	3.9	10.1

The photocell was used with a 200-ohm resistor across its terminals and the voltage drop across the resistor was read as the photometer output.

The source of radiant energy was a Westinghouse 150-watt tungsten flood lamp maintained at 110 volts plus or minus 0.5 per cent throughout. The results of the calibration may be seen in Tables I and II.

TABLE II

Calibration of photometer with Corning glass color filters showing equalization of intensity

Filter	Photometer μv.	Thermopile μv.	Energy μv. cm.$^{-2}$	Photometer sensitivity μv./μw. cm.$^{-2}$
Corning				
1–02 with Pyrex	315	.32	2.9	108
1–05 with Wratten filter .3	260	.31	2.9	84
5–77 with Wratten filter .2	147	.30	2.7	54
5–75 with Wratten filter .1	32	.30	2.7	11.8

The thermopile outputs were read with an accuracy of 5%. The calibration of the reference thermopile reproduces the International Pyroheliometric Scale of radiation to about 1%. The photocell outputs were read to within 1%. The precision of the above readings (*i.e.*, repeatability) is better than 5%.

Recalibration of the photocell at the Eppley Laboratories after completion of the experiments showed that no significant changes in energy had taken place during the course of the experiments, and therefore equal light intensity had been maintained.

Spectral sensitivity of animals

A special aquarium, 25" × 18", was constructed with a depth of 10 inches. The bottom of the aquarium was of one-quarter-inch plate glass, making it possible to measure light at the bottom of the aquarium. One side was also one-quarter-inch plate glass, to permit observation of the animals during the experiment. This side of the aquarium was covered with a cloth that excluded all extraneous light and served as a hood for the observer during experiments.

The wooden lid of the aquarium included a circular piece 15 inches in diameter, through which holes had been cut in a circular pattern, for the insertion of color filters. With this arrangement, the experimental lamp (centered above the wheel at a height of 15⅛ inches and regulated at 110 volts with a variable transformer) projected down into the water a circular pattern of colored beams, permitting the animals to choose between any of the colored beams and darkness. The wheel was movable and the light beams could be rotated.

Four color filters were used: red, blue, and two shades of green (Table I). Unfortunately, at the time, it was not possible to utilize other filters which transmitted an accurately measurable amount of energy under the experimental conditions. To avoid confusion, green filter 105 (515 mμ) will be referred to as blue-green and green filter 102 (546–559 mμ) as green.

The experiments were conducted at a constant temperature of 19° C. and usually 70–75 animals were placed in the aquarium. After the mysids had remained in darkness for measured periods of time, the experimental light was turned on and every 30 seconds, for ten minutes, the number of individuals congregated in each beam of colored light was counted. Usually another 10-minute experiment was conducted directly after this, wherein the neutral density filters were removed from the three filters containing the lowest numbers of mysids, thereby increasing the energy passing through these three filters. In this way it was possible to observe whether the animals changed their behavior pattern when intensities were greater.

During the course of the experiments, the uniformity of the light intensity was checked by measurement with a Leeds and Northrup K2 potentiometer and the Weston photronic cell.

Phototaxis

Phototactic response of *N. americana* was studied by the method employed by Beeton (1959) in his observations of *Mysis relicta*. Six individuals were placed in a 24-inch glass tube of one-inch diameter, lying horizontally to eliminate any gravitational effects. The experimental light, a 7C7 General Electric lamp, was suspended one foot above the midpoint of the tube (for spectral distribution of lamp, see Beeton, 1959; p. 205, Fig. 1). "After the mysids had been subjected for measured intervals to total darkness or light, one-half of the tube was shaded and the number of mysids in the unshaded half of the tube were recorded at 30-second intervals for a five-minute period. First the right and then the left half of the tube was shaded to detect any bias in the mysid distribution. Control runs with neither half being shaded were made at frequent intervals," Beeton (1959, p. 206).

Biological clocks

Experiments were conducted to determine whether or not *N. americana* would continue to migrate if light stimulation was removed. The observations were made in Plexiglas tubes with an inside diameter of 7.6 cm. Tubes of two lengths were used (one meter and one-half meter) and these could be joined together to obtain greater depth with the use of "O" rings and brass ring nuts and bolts.

Mysids were kept in the dark at a constant temperature and observed around the period of sunset to determine whether or not they would rise to the surface of the water in the tube, as would be expected if a biological time clock were functioning. The animals were viewed through a U. S. Army snooper scope using an infra-red light source (Baylor, 1959). Preliminary observations indicated that mysids were quite insensitive to the red region of the spectrum and therefore the important prerequisite that the experimental animal be unaffected by the light source could be fulfilled.

Results

Spectral sensitivity

In each 30-second interval the total number of mysids counted in all four color beams averaged 10 to 15, the rest remaining in the darkened portion of the tank or in the periphery of the light beams. Only those considered to be within beams were counted. Of those animals which were photopositive, a significant number stationed themselves in the blue-green light beam, the animals in this beam usually outnumbering those in the next most densely occupied beam by approximately 2 to 1 (Table III). Fewer mysids were attracted to light passing through the green and the blue filters, while the red beam attracted the least number of animals. The same order and ratio prevailed regardless of the position of the projected beams in the tank; even as the wheel containing the filters was rotated, mysids could be seen following their respective beams. The neutral density filters were then removed from the green, blue, and red color filters, increasing the energy of these beams above that of the blue-green (see Tables I and II). When this was done, the same 2 to 1 preference for the blue-green was maintained, although there was a slight increase in numbers in the blue beam and a further decrease in numbers in the red.

The Kolmogorov-Smirnov one-sample test was used to determine the significance of the results. This is a test of goodness of fit and is concerned with the degree of agreement between the distribution of a set of sample values (observed scores) and some specified theoretical distribution. It determines whether the scores in the sample can reasonably be thought to have come from a population having a theoretical distribution (Siegel, 1956). This nonparametric technique was selected because it is more powerful than the Chi Square test when there is a continuous variable and the sample is small. D values represent maximum deviation, and in each experiment they show that the distribution of animals was non-random and that the animals showed significant preferences for different colors.

Reaction towards the colored lights remained the same regardless of the time of day or the number of hours the mysids were kept in the dark. In experimental runs in which animals were kept in the dark for over 12 hours, few could be seen

TABLE III

Spectral sensitivity of N. americana

EST Time	Time in dark (hr.)	Number counted in each 10-min. period:				Total # mysids in tank	5% level D
		Red (610 mμ)	Blue (460 mμ)	Green (546–559 mμ)	Blue-green (515 mμ)		
0930	½	12	29	28	71	75	.257
*		9	35	32	69	75	.221
1200	¾	11	26	34	72	75	.258
*		8	32	30	76	75	.226
1022	1	19	31	49	78	75	.215
*		10	41	43	76	75	.200
1015	1¼	17	37	40	75	73	.195
*		10	36	35	79	73	.244
1415	1¼	35	44	36	81	75	.163
1535	1½	27	43	46	71	71	.144
*		13	52	44	87	71	.194
1607	1¾	45	59	61	107	73	.142
2120	2	21	38	44	99	72	.240
*		12	39	41	99	72	.272
1230	2½	15	35	36	70	71	.198
*		11	47	47	75	71	.189
1423	2¾	20	27	35	65	70	.193
1543	4	35	46	58	104	72	.178
2125	5	35	58	48	94	69	.150
1023	6	29	52	50	93	74	.120
*		11	42	48	92	74	.227
1642	7	24	30	41	102	67	.264
*		17	56	37	88	67	.189
2215	8	13	23	34	67	46	.233
*		5	34	29	65	46	.233
0906	10½	16	32	31	64	41	.203
*		8	30	28	66	41	.250
0853	11½	36	47	51	88	65	.148
1234	14	24	44	50	81	62	.161
1113	17½	16	28	29	49	70	.151
1523	18½	24	34	33	53	60	.117
0943	21¾	15	28	24	55	48	.200
1757	22½	25	34	26	61	69	.168
1602	26	18	33	45	81	53	.209
*		11	36	28	78	53	.255
1100	42½	9	20	19	53	40	.168

* Indicates experiments conducted with neutral density filters removed from red, blue, and green color filters.

in the light beams for the first three minutes, indicating good agreement with phototactic experiments. It is also interesting to note that copepods, which were present in the tank as food for the mysids, exhibited generally the same behavior towards the color beams in both types of experiments.

When unfiltered light from the experimental lamp was permitted to enter the water, all of the mysids in the colored beams were attracted to this white light of much greater intensity. If, however, this white light was then reduced to 4.5

microwatts by interposing neutral density filters, the animals again showed a preference for the blue-green beam, despite the fact that the energy of the white light was greater.

TABLE IV

Phototactic response of N. americana *after periods in light and total darkness*

EST Time	Dark exposure (hrs.)	Light exposure (hrs.)	Numbers in tube		Chi Square
			Shaded	Unshaded	
1253		¼	22	38	4.27*
2148		¼	13	47	19.27*
1347		¼	13	47	19.27*
1500		1	16	44	13.06*
1555		1	8	52	32.36*
1314	1		21	39	5.40*
2110	1½		6	54	38.40*
1629	2		12	48	21.60*
1945	3		23	37	3.27
1122		3	15	45	15.00*
2301	3½		31	29	.067
1944	4		26	34	1.067
2400	4		34	26	1.067
1917	5½		18	42	9.60*
1437	5½		35	25	1.67
2136	8½		9	41	20.48*
0856	11½		30	30	.000
2259	12½		38	22	4.27*
1116	13		32	18	3.91*
2206	13		32	18	3.91*
0850	14½		39	21	5.40*
1343	15½		31	19	2.88
0900		17	9	31	12.10*
0902	22		38	22	4.27*
1111	23½		31	29	.067
1405	23½		33	27	.900
2145	45½		31	29	.067
		Control runs			
0859			30	30	.000
1117			30	30	.000
1124			30	30	.000
1320			33	27	.900
1945			35	25	1.67
1952			29	31	.067
2116			35	25	1.67
2142			31	29	.067
2211			28	32	.13
2307			37	23	3.27
0013			28	32	.13

* Indicates significant Chi Square values at 5% level. Controls were run as frequently as possible after each experiment.

Phototaxis

Six mysids were placed in the horizontal tube and subjected for measured intervals to total darkness or light. One-half of the tube was shaded and the numbers of mysids in the unshaded half were recorded at 30-second intervals for a five-minute period.

Significant differences in distribution were never found in control runs, but were found when one-half of the tube was shaded after the mysids had been in light or dark for a period of time. Mysids were photopositive unless they had been subjected to total darkness for 12 hours; after longer periods in the dark they were photonegative (Table IV). The photonegative condition lasted only for a short time, as they became light-adapted within 3 to 5 minutes of exposure to light. Beeton (1959) found in his laboratory experiments that *Mysis relicta* could be photonegative in the morning and also in the evening, and he stated that it was not likely that the photic response had a persistent diurnal rhythm. The same is true of *N. americana,* since in both the evening and the morning it could become photonegative if kept in the dark for over 12 hours.

Experiments also revealed that mysids which were photopositive could be made to move into the shaded area of the tube if the intensity of the light was increased. This agrees with the results obtained by other workers (Johnson, 1938; Beeton, 1959).

Biological clocks

Examination of mysids kept in total darkness in Plexiglas tubes revealed no significant movement towards the surface at the time of day when the animals in the Bay were ascending. Usually the mysids remained equally distributed throughout the tube at all times.

Discussion

In spectral sensitivity experimentation on mysids, the experimental animals have not previously been offered a choice of lights of different wave-lengths of the same intensity. Hess (1910) worked with *Mesopodopsis slabberi* and found that if these mysids were kept for a time in the dark, and then brought into the light, all of the animals swam rapidly towards the source of the light. When a spectrum was passed through the tank, they rapidly congregated in the yellow-green region and remained there. Since the relative intensities of the different parts of the spectrum were neither controlled nor measured, the animals' apparent preference for the yellow-green may have been due to differences in intensity.

Beeton (1959), experimenting with *Mysis relicta,* measured the response of animals in an aquarium to an experimental light which was passed through different combinations of Corning glass color filters and neutral density filters. He mathematically calculated the total energy output of each filter combination, using the per cent transmission of the color filter and the distribution curve of spectral energy of the experimental lamp. He determined that *M. relicta* showed greatest sensitivity at wave-lengths in the vicinity of 515 mμ and 395 mμ.

Results of spectral sensitivity experiments on *N. americana* indicate a distinct preference for light having a wave-length of 515 mμ. The yellow-green light

which attracted Hess' *Mesopodopsis* is closely approximated by the green filter 102 (546–559 mμ), but *N. americana* showed no preference for this under controlled intensity conditions.

The experiments were conducted at a temperature of 19° C. and it would have been desirable to repeat them at lower temperatures, since 19° C. is near the annual maximum in Narragansett Bay.

The comparatively high temperatures may have been one of the reasons why relatively few of the mysids in the experimental tank were attracted to the light beam. On the other hand, the field results of this study indicate that not all of the mysids undergo vertical migration, some animals remaining on the bottom during the night throughout the year. Thus there must be other physiological mechansims operating both in the field and in the laboratory, which are responsible for keeping certain members of the population from responding to light by migrating vertically.

In the spectral sensitivity experiments it was shown that mysids still preferred the blue-green light beam even when the intensity of the other colored light beams was greater. *N. americana* is capable of distinguishing between colors differing in wave-band maxima by only 31–35 mμ, and shows a distinct preference for one of these. Even where greater intensity is present, the mysids seek out this blue-green light. When unfiltered light from the experimental lamp was passed into the aquarium, the mysids quickly congregated in this light, deserting all of the color beams. However, when this light was adjusted with Wratten neutral density filters (4.5 microwatts $cm.^{-2}$), reducing its intensity to a level which was still above that of the colored beams, the mysids again showed a preference for the blue-green. According to John Roche of the Eppley Laboratories (personal communication), when the unfiltered light from the experimental lamp was projected into the tank, more light of the wave-length 515 mμ was present in this beam than in the blue-green beam, but when this white light was reduced with neutral density filters, less light of 515 mμ was present in the white beam than in the blue-green beam. In each case the mysids congregated in that beam transmitting the greatest amount of energy of the wave-length 515 mμ.

Experiments indicate that it is not likely that the photic response in *N. americana* is governed by a biological time clock. Experiments also revealed that 12 hours in continuous darkness are required to make this species photonegative. The significance of these findings in regard to the vertical migration of *N. americana* will be discussed in a subsequent publication.

Dr. David M. Pratt reviewed the manuscript and Mr. Theodore A. Napora gave valuable assistance during the experiments. Calibration of equipment and test runs of the entire experimental apparatus were conducted at the Eppley Laboratories, Newport, Rhode Island.

Summary

1. Laboratory experiments were conducted to determine the spectral sensitivity of *N. americana*. The intensity of light beams passing through four Corning glass color filters was made equal with Wratten neutral density filters.

2. The positively phototactic animals showed a definite preference for light passing through a color filter having peak transmission at 515 mμ.

3. Increasing the intensity of light passing through the three other color filters did not alter the mysid preference for the wave-length 515 mμ.

4. Phototactic experiments revealed that *N. americana* was photopositive unless subjected to total darkness for 12 hours; after longer periods in the dark they were photonegative.

5. Experiments indicate that it is not likely that the photic response in *N. americana* is governed by a biological time clock.

LITERATURE CITED

BAYLOR, E. R., 1959. Infra-red observations and cinematography of Microcrustacea. *Limnol. Oceanogr.*, **4**: 498–499.

BEETON, A. M., 1959. Photoreception in the opossum shrimp, *Mysis relicta* Lovén. *Biol. Bull.*, **116**: 204–216.

CUSHING, D. H., 1951. The vertical migration of planktonic Crustacea. *Biol. Rev.*, **26**: 158–192.

HESS, C., 1910. Neue Untersuchungen über den Lichtsinn bei wirbellosen Tieren. *Pflueger's Arch.*, **136**: 282–367.

HULBURT, E. M., 1957. The distribution of *Neomysis americana* in the estuary of the Delaware River. *Limnol. Oceanogr.*, **2**: 1–11.

JOHNSON, W. H., 1938. The effect of light on the vertical movements of *Acartia clausii* (Giesbrecht). *Biol. Bull.*, **75**: 106–118.

SIEGEL, S., 1956. Non Parametric Statistics for the Behavioural Sciences. McGraw-Hill, New York, 312 pp.

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